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Preparation of highly selective stationary phases for high-performance liquid chromatographic separation of enantiomers by direct copolymerization of monomers with single or twin chiral ligands

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Abstract

Uniformly sized macroporous polymer beads, which can be used as chiral stationary phase (CSP), have been prepared by the staged templated suspension polymerization process using chiral monomer as one of the copolymerization components. This approach enables the preparation of CSPs for which properties such as pore size, pore volume, surface area, chemistry, and chiral ligands can be tuned over a broad range. Several types of well-defined chiral monomers were prepared and allowed to assess synergistic effect of multiple selectors attached to a branched linker as well as the effect of the length and chemistry of the linker. Microscale batch screening was used for simple and rapid evaluation of selectivity. The most promising candidate CSPs were prepared on a larger scale and packed into HPLC columns. Their performance was demonstrated on the separation of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid alkylamides. The highest separation factors α of up to 27 were observed for CSPs prepared from monomers containing the branched spacer. These highly selective CSPs also enabled the separation of larger amounts of the target racemates upon column overload conditions.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Monomers

1. Introduction

The production of single enantiomers is an important target for the pharmaceutical, agrochemical, and food industry today. Although many chiral compounds enriched with one enantiomer can be prepared directly from chiral precursors or by means of asymmetric synthesis [1], the resolution of mixtures of both enantiomers remains an important technique that affords the required single enantiomers in high purity. A number of methods such as crystallization, distillation, enantioselective membrane techniques, and chromatography are currently available to achieve the desired separation of individual enantiomers from their mixtures [2–5]. The chromatographic methods include high-performance liquid chromatography and gas chromatography, as well as electrochromatography and electrophoresis.

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These techniques, characterized by the use of chiral stationary phases (CSPs) or chiral additives are extremely valuable for analytical assays in both academic research and clinical laboratories. Because of its high efficiency and ease of operation, chromatography can be used virtually on any scale, including preparative and production scale separations. For example, the preparative liquid chromatography recently implemented in simulated moving bed mode enables the annual production of up to 50 metric tons of pure enantiomers [6]. Therefore, the quantities produced by these chromatographic separations are sufficient not only to carry out early pharmacological and toxicological studies, providing both enantiomers for comparative biological testing, but also to satisfy the commercial demand for many drugs. Since chromatography also continues to be used for both process and quality control, as well as for final product purification of the compounds obtained via the larger scale chiral technology, current targets in enantioselective chromatography include both highly efficient analysis, with the ultimate goal of rapid, real time monitoring and control, and large-scale downstream processing of both racemic and enantiomerically enriched products.

The pioneering studies of Allenmark, Armstrong, Blaschke, Cram, Davankov, Hermansson, Okamoto, Pirkle, and others have led to a number of different chiral stationary phases (CSPs) for liquid chromatography useful in the separation of enantiomers. Their number currently exceeds 1000 [7] with more than 100 commercially available. This explosive growth stems inter alia from the fact that the determination of the enantiomeric composition of chiral drugs and the pharmacokinetics as well as metabolic fate of each enantiomer have become important issues in their development, regulation, and clinical use [8].

Chiral separation media are generally quite complex: they typically involve the solid support, the selector, and a linker. As a result of advantages such as its commercial availability in a large variety of types, relatively low cost, resistance to swelling, and good column efficiency, silica is the most common chromatographic support for CSPs. The specially designed synthetic polymer supports with well-controlled surface chemistries we have introduced recently may provide a viable alternative to silica for the preparation of highly selective chiral separation media [9]. Once the support is available, the selector is typically chemically attached or coated on the pore surface of the preformed beads. In an alternative approach that has been developed in our laboratory [10], chiral monomer can be added to the suspension polymerization system during the preparation of porous monodisperse beads [11,12]. Broader application of this technique has yet to be demonstrated.

While most approaches to polymeric CSPs involve the chemical modification of pre-formed beads [13], we now report a polymerization approach in which monomers containing the chiral selector moiety are polymerized to form monodisperse macroporous beads.

2. Experimental

2.1. Chemicals

2.1.1. Boc-L-proline-indananilide (1)

A 500-ml three-neck round bottom flask was charged with Boc-L-proline (32.3 g, 150 mmol) and CH_2Cl_2 (300 ml) and cooled in an ice bath to 0°C. Then ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ, 37.5 g, 152 mmol) was added. A solution of 5-aminoindan (20 g, 150 mmol) in CH_2Cl_2 (100 ml) was dropwise added over a period of 45 min. The reaction mixture was stirred at 0°C for 2 h, at room temperature (rt) for another 10 h, then washed with 1 mol/l HCl (2×100 ml), saturated $NaHCO_3$ (2×100 ml), water (150 ml), brine (2×100 ml), and dried over $MgSO_4$. Evaporation of the solvent and crystallization of the crude product from ethyl acetate–CH_2Cl_2 (1:1) afforded **1** as a white crystalline solid (42 g, 86%); m.p.: 148–149°C.

2.1.2. L-Proline-5-indananilide (2)

A 1:1 mixture of TFA and acetic acid (80 ml) kept at 0°C was added dropwise to a solution of **1** (30 g, 91 mmol) in CH_2Cl_2 (150 ml) over a period of 90 min. The reaction mixture was then stirred at room temperature for ca. 15 h with TLC monitoring, then the organic solvent was evaporated in vacuum and the residue diluted with 100 ml CH_2Cl_2. Aqueous 1

mol/l KOH was slowly added to this solution under vigorous mixing until the pH of the aqueous layer reached 9–10. Two layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2×70 ml). The combined organic phase was washed with water (200 ml), brine (200 ml) and dried over $MgSO_4$. Evaporation of the solvent afforded light brown oil (21 g, 100%) which was used without further purification in the subsequent reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43–1.74 (m, 2H), 2.02–2.27 (m, 4H), 2.24 (s, 1H), 2.83–3.05 (m, 6H), 3.78–3.83 (dd, J=9.2, 5.2 Hz, 1H), 7.13–7.60 (m, 2H), 7.96 (s, 1H), 9.67 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.7, 26.3, 30.8, 32.4, 33.1, 47.4, 61.1, 115.7, 117.4, 124.4, 136.1, 139.8, 145.1, 173.3. LC–MS: 231 (M+H).

*2.1.3. L-4-[2-(indan-5-ylcarbamoyl)-pyrroline-1-yl]-4-oxo-butyric acid (**3**)*

A 300-ml two-neck flask was charged with **2** (20 g, 86 mmol), succinic anhydride (12.1 g, 121 mmol), dimethylaminopyridine (DMAP, 1 g, 8.6 mmol, 0.1 equiv.) and a 1:1 triethylamine/THF mixture (200 ml). The partially miscible reaction mixture was refluxed at 70°C for 5 h. During this time, the color of the solution turned dark. After evaporating the solvent, the residue was dissolved in ethyl acetate (200 ml) and 1 mol/l HCl was slowly added until the solution reached a pH value of 2–3. After extraction of the aqueous phase with ethyl acetate (2×100 ml), the combined organic phase was dried over $MgSO_4$. Concentration of the solution by rotary evaporator afforded a suspension, from which a whitish solid **3** (22 g) was recovered by filtration. Further concentration and filtration afforded another 2.5 g of product for an overall yield of 87%; m.p.: 193–195°C. ^{1}H NMR (d_4-methanol, 400 MHz): δ 1.83–2.02 (m, 6H), 2.16–2.20 (m, 1H), 2.38–2.61 (m, 4H), 3.46–3.56 (m, 1H), 3.56–3.65 (m, 1H) 4.36–4.54 (m, 1H), 6.92–7.04 (d, 1H, J=6 Hz), 7.08–7.12 (d, 1H, J=6 Hz), 7.33 (s, 1H), 8.96 (s, 1H). ^{13}C NMR (d_4-methanol, 125 MHz): δ 24.70, 25.51, 27.75, 29.04, 29.60, 32.07, 32.86, 47.54, 60.71, 116.15, 117.94, 124.12, 136.16, 139.75, 144.74, 169.20, 172.18, 176.72. FT-IR (KBr): ν 2970 cm^{-1} (COOH,1726 cm^{-1} (C=O). HRFABMS Calc. 331.2 (M^+), Found 331.2.

*2.1.4. 4-Oxo-4-[2-(2,3,3a,4-tetrahydro-1H-inden-5-ylcarbamoyl)-pyrrolidin-1-yl]-butyric acid 2-(methacryloyloxy)-ethyl ester (**4**)*

A 250-ml flask was charged with the acid **3** (5 g, 15 mmol) and DMAP (183 mg, 1.5 mmol) in CH_2Cl_2 (50 ml). Then a solution of 2-hydroxyethyl methacrylate (HEMA, 2.1 g, 16 mmol) in CH_2Cl_2 (20 ml) was added slowly at 0°C under N_2 atmosphere. Dicyclohexylcarbodiimide (DCC) was added in a single portion and the mixture was stirred for 1 h at 0°C and 10 h at room temperature. Floating precipitate of dicyclohexylurea was filtered off and the filtrate washed with saturated $NaHCO_3$ (30 ml), water (30 ml), brine (30 ml), and dried overnight. Evaporation of the solvent of purification of the crude product using a silica gel column and ethyl acetate–hexane (3:2) as an eluent afforded **4** as a light brown oil (6.7 g, 100%) which later solidified in freezer to a white solid; m.p.: 50–52°C.^{1}H NMR (400 MHz, $CDCl_3$): δ 1.94 (s, 3H), 1.98–2.08 (m, 4H), 2.17–2.21 (br m, 1H), 2.48 (br s, 1H), 2.63–2.82 (m, 4H), 3.49–3.56 (m, 1H), 3.65–3.70 (m, 1H), 4.34 (s, 2H), 4.73 (br s, 1H), 5.60 (s, 1H), 6.13 (s, 1H), 7.06 (d, J=4 Hz, 1H), 7.21 (d, J=8 Hz, 1H), 7.60 (s, 1H), 9.33 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2, 18.3, 21.05, 25.0, 25.6, 29.2, 32.3, 32.9, 47.5, 60.7, 62.3, 116.1, 117.9, 124.1, 126.1, 135.9, 136.9, 139.6, 144.7, 167.0, 169.1, 1171.1, 171.8. LC–MS: 465 (M+Na).

*2.1.5. Succinic acid benzyl ester 2-hydroxyethyl ester (**5**)*

To a stirred solution of ethylene glycol (1.8 g, 30 mmol), succinic acid monobenzyl ester **5a** (832 mg, 4 mmol) and DMAP (97 mg, 0.8 mmol) in CH_2Cl_2 (50 ml) was added DCC (886 mg, 4.4 mmol) in a single portion at 0°C under N_2 atmosphere. The reaction mixture was stirred at 0°C for 1 h and at room temperature for 10 h and the dicyclohexylurea was filtered. The filtrate was washed with water, brine, and dried over $MgSO_4$. Evaporation of the solvent and purification of the crude product by column chromatography (silica gel, 2% methanol–CH_2Cl_2) afforded colorless oil of **5** (720 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64–2.75 (m, 4H), 2.89 (t, J=4 Hz, 1H), 3.75 (q, J=5 Hz, 2H), 4.19 (t, J=4 Hz, 2H), 5.12 (s, 2H), 7.27–7.37 (m, 5H). ^{13}C

NMR (125 MHz, $CDCl_3$): δ 28.9, 29.2, 60.7, 66.3, 66.6, 128.2, 128.3, 128.6, 135.6, 172.4, 172.5. LC–MS: 275 (M+Na).

*2.1.6. Succinic acid benzyl ester 2-{4-[2-(indan-5-ylcarbamoyl)-pyrrolidin-1-yl]-4-oxo-butyryloxy}-ethyl ester (**6**)*

To a stirred solution of acid **3** (1 g, 3 mmol), alcohol **5** (756 mg, 3 mmol) and DMAP (33 mg, 0.3 mmol) in CH_2Cl_2 (6 ml) was added DCC (618 mg, 3 mmol) at 0°C under N_2 atmosphere. The reaction mixture was stirred at room temperature for 17 h. After the solid dicyclohexylurea was removed by filtration, the filtrate was diluted with CH_2Cl_2 (15 ml) and washed with saturated $NaHCO_3$ solution (30 ml), water (30 ml), brine (30 ml) and dried over $MgSO_4$. Evaporation of the solvent and purification of the crude product by column chromatography (silica gel, ethyl acetate–hexane 3:1) yields colorless oil **6** (1.52 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.96–2.06 (m, 4H), 2.61 (m, 1H), 2.65 (m, 4H), 4.72 (d, J=6 Hz, 1H), 5.12 (s, 2H), 7.05 (1/2 ABq, J=9 Hz, 1H), 7.13 (1/2 ABq, J=9 Hz, 1H), 7.33 (s, 5H), 7.58 (s, 1H), 9.27 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.9, 25.6, 28.94, 28.97 29.07, 29.1, 32.3, 32.9, 47.6, 60.4, 60.7, 62.3, 62.4, 66.5, 116.1, 117.9, 124.21, 128.2, 128.3, 128.6, 135.8, 136.5, 139.6, 144.7, 169.1, 171.9, 172.0, 172.9. LC–MS: 587 (M+Na).

*2.1.7. Succinic acid mono-(2-{4-[2-(indan-5-ylcarbamoyl)-pyrrolidin-1-yl]-4-oxo-butyryl oxy}-ethyl) ester (**7**)*

A solution of benzyl ester **6** (1.5 g, 2.67 mmol), Pd/C (100 mg) in ethyl acetate (6 ml) was stirred in H_2 atmosphere at room temperature for 10 h. The reaction mixture was filtered and the filtrate vacuum dried to give **7** as a colorless liquid (1.24 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97–2.05 (m, 4H), 2.12–2.52 (m, 2H), 2.59–2.65 (m, 4H), 2.67–2.85 (m, 8H), 3.52–3.69 (m, 2H), 4.13–4.30 (m, 4H), 4.72 (d, J=6 Hz, 1H), 7.08 (1.2 ABq, J=9 Hz, 1H), 7.16 (1/2 ABq, J=9 Hz, 1H), 7.44 (s, 1H), 9.08 (s, 1H), 10.31 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.4, 22.5, 25.3, 28.5, 28.6, 28.8, 32.0, 32.6, 47.3, 60.1, 61.8, 62.1, 115.9, 117.7, 123.7, 135.2, 139.3, 144.2, 169.8, 171.5, 171.8, 172.7, 174.8.

*2.1.8. Succinic acid 2-{4-[2-(indan-5-ylcarbamoyl)-pyrrolidin-1-yl]-4-oxo-butyryloxy}-ethyl ester 2-(2-methacryloyloxy)ethyl ester (**8**)*

To a stirred solution of **7** (1.5 g, 2.6 mmol), and DMAP (31.2 mg, 0.25 mmol) in CH_2Cl_2 (5 ml) was added 2-hydroxyethyl methacrylate (332 mg, 2.56 mmol) at 0°C under N_2 followed by the addition of DCC (580 mg, 2.82 mmol) and the reaction mixture was stirred for 2 h at 0°C followed by 10 h at room temperature. After filtration of the mixture and concentration and purification of the filtrate by column chromatography (silica gel, ethyl acetate–hexane 3:1) 1.5 g of colorless oil **8** (100%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.89 (s, 3H), 1.93–2.09 (m, 4H), 2.49 (m, 2H), 2.63 (s, 4H), 2.72–2.84 (m, 4H), 4.72 (d, J=9 Hz, 1H), 5.58 (s, 1H), 6.11 (s, 1H), 7.06 (1/2 ABq, J=6 Hz, 1H), 7.15 (1/2 ABq, J=6 Hz, 1H), 7.43 (s, 1H), 9.23 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2, 18.2, 21.0, 24.9, 25.6, 27.6, 28.8, 28.9, 29.1, 32.3, 32.9, 47.5, 60.4, 60.7, 62.4, 116.1, 117.8, 124.1, 126.1, 135.9, 136.5, 139.6, 144.7, 167.1, 169.1, 171.8, 171.9, 172.9.

*2.1.9. 4-[2-(Indan-5-ylcarbamoyl)-pyrrolidin-1-yl]4-oxo-butyric acid-2-carboxy-3-{4-[2-(indan-5-yl-carbamoyl)-pyrrolidin-1-yl]-4-oxo-butyryloxy}-2-methyl-propyl ester (**9**)*

To a mixture of 3-hydroxy-2-hydroxymethyl-2-methyl-propionic acid benzyl ester (0.50 g, 2.23 mmol), **3** (1.53 g, 4.62 mmol) and DPTS (0.37 g, 1.24 mmol) in CH_2Cl_2 (ca. 30 ml), was added DCC (1.38 g, 6.69 mmol). The reaction mixture was stirred under inert atmosphere at room temperature overnight, before it was filtered and concentrated. The crude reaction mixture was subjected to flash column chromatography to yield the precursor of **9** (1.90 g, 100%) as a beige crystalline solid. Then, a mixture of the obtained compound (1.00 g, 1.18 mmol) and Pd/C (0.096 g) in ethyl acetate (20 ml) was stirred under H_2 overnight, filtered, and concentrated to give **9** (0.893 g, 99.7%) as a white crystalline solid. ^{1}H NMR (d_1-chloroform, 400 Hz): δ 1.22 (s, 3H), 1.98–2.06 (m, 12H), 2.49–2.85 (m, 20H), 3.38–3.45 (m, 2H), 3.46–3.59 (m, 2H), 4.68–4.71 (m, 2H), 7.07 (d, J=8.1 Hz, 2H), 7.20 (d, J=8.0 Hz, 2H), 7.39–7.45 (m, 2H), 9.14–9.20 (m,

2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.68, 24.80, 25.55, 27.15, 28.87, 29.09, 32.29, 32.94, 34.90, 46.26, 47.47, 60.68, 65.47, 66.79, 116.05, 117.83, 124.22, 128.08, 128.34, 128.56, 135.57, 136.39, 139.71, 144.84, 169.38, 168.89, 171.64, 171.80, 172.19, 172.49, 174.72.

*2.1.10. Dendritic monomer (**10**)*

The preparation is essentially identical to that of **4** using acid **9** (3.5 g, 4.6 mmol) and 2-hydroxyethyl methacrylate (717 mg, 5.5 mmol). The product was purified by a silica gel column chromatography using 3–5% MeOH/CH_2Cl_2 mixture to obtain **10** as beige solid (3.65 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (s, 3H), 1.29 (s, 3H), 1.91–2.10 (m, 16H), 2.24–2.84 (m, 20H), 3.46–3.71 (m, 4H), 4.22–4.37 (m, 8H), 4.68–4.73 (m, 2H), 5.57 (s, 1H), 6.10 (s, 1H), 7.07 (d, J=6, 2H), 7.14 (d, J=6, 2H), 7.44 (s, 2H), 9.11–9.22 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.8, 18.3, 24.9, 25.7, 27.5, 32.4, 33.1, 46.3, 48.9, 60.8 (2C), 62.2, 62.8, 65.4, 116.1, 117.9, 124.3, 126.3, 135.9, 136.5, 139.8, 144.9, 167.1, 169.1, 171.8, 172.1, 172.6. LC–MS: 895 (M+Na).

*2.1.11. 2-Acetoxymethyl-3-hydroxy-2-methyl-propionic acid benzyl ester (**11**)*

To a mixture of 2-(hydroxymethyl)-2-methyl-5-oxohexanoate (3.00 g, 13.38 mmol) and DMAP (0.026 g, 0.213 mmol) in THF (20 ml) at 0°C, was added pyridine (0.238 ml, 2.96 mmol) and acetic anhydride (0.252 ml, 2.68 mmol). The reaction mixture was allowed to slowly warm to room temperature, and was stirred overnight. The solvent was evaporated and the crude material purified using flash column chromatography (ethyl acetate/hexanes, 3:2) to give **11** (0.70 g) as colorless oil. ^{1}H NMR (500.1 MHz, chloroform-d_1): δ 1.23 (s, 3 H), 2.00 (s, 3H), 3.67 (d, J=12 Hz, 1H), 3.73 (d, J=12 Hz, 1H), 4.22 (d, J=11 Hz, 1H), 4.33 (d, J=11 Hz, 1H), 5.16 (d, J=12 Hz, 1H), 5.20 (d, J=12 Hz, 1H), 7.33–7.38 (m, 5H). MS TOF ES$^+$: 289.14 (Na^+).

*2.1.12. 4-[2-(Indan-5-ylcarbamoyl)-pyrrolidin-1-yl]-4-oxo-butyric acid-3-acetoxy-2-carboxy-2-methyl-propyl ester (**12**)*

To a CH_2Cl_2 solution (40 ml) of **11** (0.70 g, 2.63 mmol), **3** (0.912 g, 2.761 mmol) and DPTS (0.155 g, 0.527 mmol) kept at 0°C was added DCC (1.1 g, 5.2 mmol). The reaction mixture was stirred under inert atmosphere at room temperature overnight, filtered, concentrated and subjected to flash column chromatography (100% ethyl acetate) to give the precursor of **12** as a colorless viscous oil (1.50 g, 99%). A methanol solution (30 ml) of the obtained oil (1.50 g, 2.60 mmol) and Pd/C (0.12 mg) was stirred under H_2 overnight, filtered, and concentrated to give **12** (1.20 g, 95%) as a colorless crystalline solid with a melting point of 150–155°C. ^{1}H NMR (500.1 MHz, chloroform-d_1): δ 1.25 (s, 3H), 1.20–1.28 (m, 6H), 1.77–1.93 (m, 4H), 2.01 (s, 3H), 2.60–2.85 (m, 4H), 3.15–3.25 (m, 1H), 3.43–3.48 (m, 1H), 3.60–3.68 (m, 1H), 4.19–4.30 (m, 4H), 4.72–4.74 (m, 1H), 7.09 (d, J=6 Hz, 1H), 7.24 (d, J=6 Hz, 1H), 7.58 (s, 1H), 9.20 (s, 1H). ^{13}C NMR (125 Hz, chloroform-d): δ 18.2, 20.9, 24.7, 24.8, 25.52, 25.6, 27.4, 29.1, 29.2, 32.2, 32.9, 33.3, 33.7, 46.1, 47.5, 49.3, 56.4, 60.7, 66.1, 66.5, 116.2, 118.0, 124.2, 136.3, 139.7, 144.8, 169.2, 170.9, 172.1, 172.6, 177.6, MS TOF ES$^-$: 487.69 (M^-).

*2.1.13. Monosubstituted dendritic monomer (**13**)*

The preparation is similar to that of **4** using acid **12** (1 g, 2.05 mmol) and 2-hydroxyethyl methacrylate (319 mg, 2.45 mmol). The product was purified by a silica gel column chromatography using 2% MeOH/CH_2Cl_2 mixture to obtain **13** as beige solid (1.2 g, 97%).^{1}H NMR (300 MHz, chloroform-d_1): δ 1.25 (s, 3H), 1.30 (s, 3H), 1.79–2.08 (m, 12H), 2.72–2.90 (m, 10 H), 3.60–3.81 (m, 4H), 4.12–4.27 (m, 6H), 4.76 (m, 1H), 5.60 (s, 1H), 6.12 (s, 1H), 7.12 (d, J=6 Hz, 1H), 7.19 (d, J=6 Hz, 1H), 7.48 (s, 1H), 9.18–9.22 (m, 1H). LC–MS: 623 (M+Na).

2.2. Preparation of uniformly sized macroporous beads

The preparation of the uniformly sized beads derived from chiral monomers **4**, **8**, **10, 13** using the staged templated suspension polymerization was carried out as described previously [14]. This process is exemplified on the preparation of CSP 15. Monodisperse polystyrene shape templates (0.75 ml, 1.5 μm, 10% dispersion in water) prepared by emulsifier-free emulsion polymerization [15] were acti-

vated by swelling with dibutyl phthalate (0.69 ml), which was emulsified in 75.0 ml of 0.25% aqueous sodium dodecylsulfate (SDS) solution. A mixture containing ethylene dimethacrylate (1.96 g), chiral monomer **10** (2.14 g), azobisisobutyronitrile (1% with respect to the total weight of monomers), and toluene (4.83 g) was emulsified by sonication in 75.0 ml of aqueous 0.25% SDS solution and added to the dispersion of activated templates in a 500-ml Erlenmeyer flask. The mixture was stirred at room temperature until the droplets of the emulsified polymerization mixture disappeared. Then, 50 ml of 4% aqueous solution poly(vinyl alcohol) (87–89% hydrolyzed, average molecular mass: 85 000–146 000, Aldrich 36308-1) was added to adjust its concentration in the system to 1%. This dispersion was purged with nitrogen for 20 min, the flask was sealed, and the polymerization carried out in flasks placed in an orbiting shaker bath set to 225 rotations/min and 75°C for 16 h. The beads formed were repeatedly decanted in water and methanol until the supernatant liquid was clear. The beads were dried in air and then extracted with toluene for 30 h and dichloromethane for 8 h to remove the linear polystyrene originating from the templates. Drying in vacuum at 50°C affords 3.5 g beads representing 85% yield.

2.3. Characterization of chiral stationary phases

2.3.1. Porous properties

The specific surface area of the beads was calculated from the isotherm of nitrogen using BET equation, and the pore size distribution in the dry state was determined from mercury intrusion porosimetry using ASAP 2010 and Auto Pore instruments, respectively (both Micromeritics).

2.3.2. Selector loading

The selector loadings of the CSPs were calculated from the nitrogen content determined by elemental analysis.

2.3.3. Microscale screening for selectivity

Various amounts of racemic *N*-(3,5-dinitrobenzoyl)-leucine diallylamide were dissolved in 1 ml of dichloromethane/hexane (80:20) and added to glass vials containing 40 mg of CSP. The vials were sealed and allowed to equilibrate for 2 h at room temperature. The supernatant liquid was collected and analyzed by HPLC using a commercial column (Chiralcel OD, 250×4.6 mm I.D., purchased from Daicel Chemical Industries Ltd.) and dichloromethane/hexane (80:20) as the mobile phase at a flow-rate of 1.0 ml/min. The peaks were detected at 254 nm.

2.3.4. Chromatography

The chiral stationary phases were slurry packed at a constant pressure of 20 MPa into 150×4.6 mm I.D. stainless steel columns. A Waters HPLC system consisting of two 510 HPLC pumps, a 717 plus autosampler, and a 486 UV detector, and controlled by Millennium 2010 software, was used for all chromatographic measurements. Chiral separations were carried out using a hexane/dichloromethane mixture as the mobile phase at a flow-rate of 1 ml/min. The selectivity factors α were calculated as a ratio of the retention factors of individual enantiomers k_1' and k_2' which are defined as $k_i' = (t_i - t_0)/t_0$ where t_i and t_0 represent the retention times of the specific enantiomer and 1,3,5-tri-*t*-butylbenzene (void volume marker), respectively. The racemic analytes, *N*-(3,5-dinitrobenzoyl)-α-amino acid alkylamides, were prepared by methods reported elsewhere [9].

3. Results and discussion

3.1. Selection of monomers

In our previous studies, we used a combinatorial approach to prepare a large number of low molecular mass selectors and attached them to specifically designed porous polymer beads to screen for CSPs with high selectivities [16]. Following deconvolution of a library-on-beads, we identified a CSP with a selector containing the L-proline-1-indananilide moiety that afforded α-values of over 23 under normal-phase HPLC conditions. Therefore, we introduced this proline-based structure in a series of chiral monomers that could be used in the preparation of bead using the staged templated suspension polymerization process (Fig. 1) (vide supra). Following the reaction path shown in Scheme 1, we first prepared **3** from 5-aminoindan and Boc-L-proline

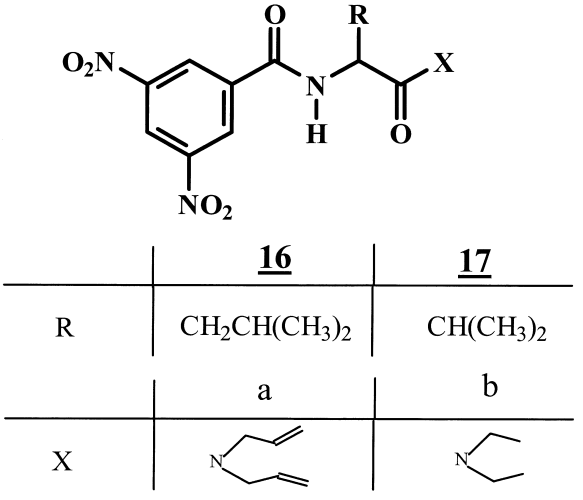


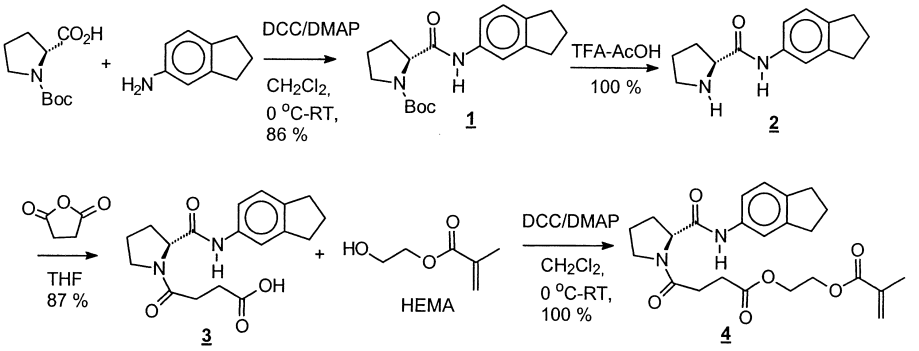
Fig. 1. Structures of analytes used for evaluation of CSPs prepared by polymerization of chiral monomers.

followed by deprotection and reaction with succinic anhydride. Monomer **4** was then prepared in quantitative yield by reaction of **3** with HEMA in the presence of DCC/DMAP in dichloromethane. In order to examine the effect of linker on selectivity of CSPs, monomer **8** that includes a short aliphatic polyester linker between the selector and the polymeric support was also prepared in excellent yield as shown in Scheme 2. Reaction of succinic acid monobenzyl ester **5a** with an excess of ethylene glycol under DCC coupling conditions affords the desired mono substituted ethylene glycol **5**. Subsequent reaction of **5** with selector **3** gives compound **6**, which is deprotected to acid **7** by hydrogenolysis using Pd/C in ethyl acetate. Finally, coupling of **7** with HEMA in the presence of DCC/DMAP affords monomer **8** in nearly quantitative yield.

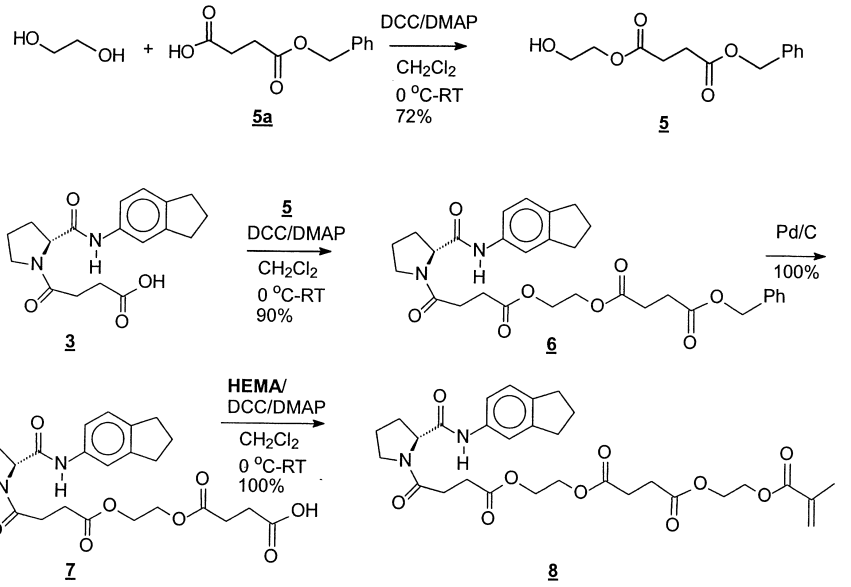
To obtain monomer **10** with twin chiral selectors, the benzyl protected 3-hydroxy-2-hydroxymethyl-2-methyl-propanoic acid was coupled to selector **3** and the product deprotected by hydrogenolysis to afford compound **9** in 75% overall yield (Scheme 3). Finally, reaction of **9** with HEMA under DCC/DMAP/CH_2Cl_2 conditions affords the desired monomer **10** in 96% yield. To assess the possible synergistic effect of twin selector moieties, we also prepared the corresponding mono-substituted chiral monomer **13** from 3-benzyloxy-2-hydroxymethyl-2-methyl-propanoic acid **11** following the procedure of Scheme 4 that was similar to that used for the preparation of monomer **10**.

3.2. Preparation of beads

Monodisperse beads with controlled size, porous properties, and surface chemistry were prepared using staged templated suspension polymerization that we have developed earlier [14,17]. This process requires the use of size monodisperse polystyrene latex particles used as templates. In the first stage, these polystyrene templates are activated by swelling with an emulsion of organic solvent such as dibutyl phthalate or 1-chlorodecane. The activated templates are swollen further in the second stage with an emulsified mixture of solvents, monomers, cross-linking agent, and free-radical initiator. Once this emulsified mixture is completely transferred into the activated templates, a solution of polyvinyl alcohol is added to act as a steric suspension stabilizer for the



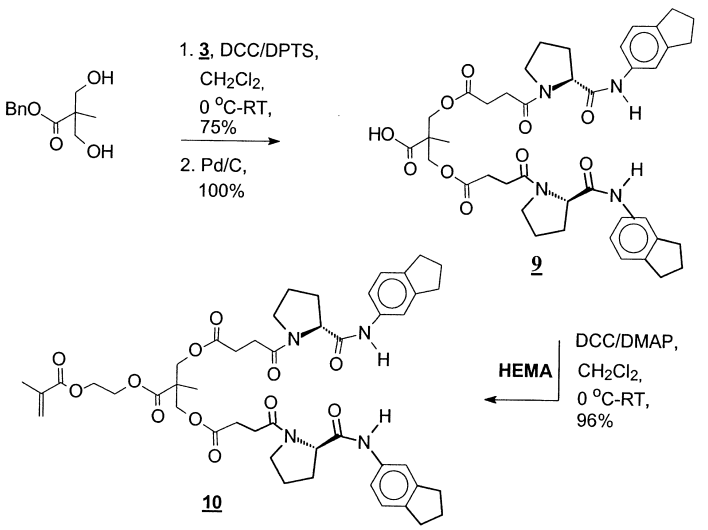
Scheme 1.



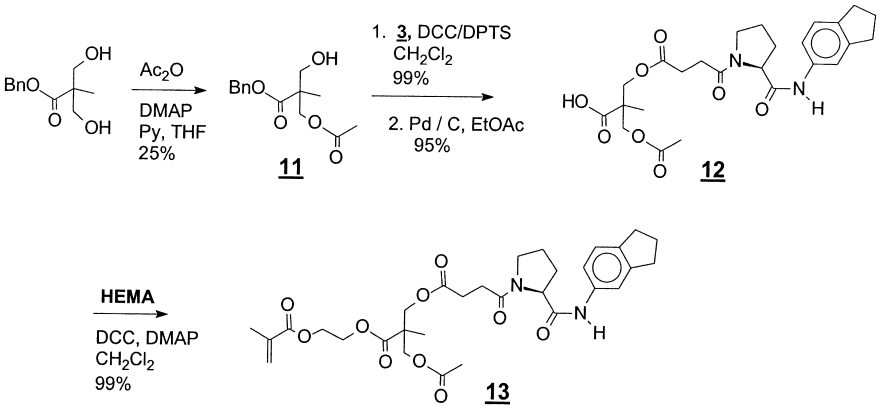
Scheme 2.

swollen templates and the polymerization process is initiated by heating to 70°C. Continuous stirring prevents coalescence of the stabilized polymerizing particles.

As indicated above, all components of the emulsified polymerization mixture must traverse the aqueous phase to enter and swell the templates. This requires that these components have sufficient affinity towards the activated templates to facilitate the transfer and keep their concentration in the aqueous phase low. The thermodynamic background of this free-energy driven process has been laid by Ugelstad [18]. Monomers commonly used in this process such as styrene and its substituted derivatives [19–21] and



Scheme 3.



Scheme 4.

glycidyl methacrylate [22] have both low solubility in water and small molecular sizes. Monomers with higher hydrophilicity such as HEMA are quite soluble in water restricting their transfer through the aqueous phase into the templates. To solve this problem, we selected a porogenic solvent that ''attracts'' the monomer into the templates to assist with transport of the monomer into the organic phase of the swollen templates [23]. This is even more critical for monomers that, in addition to their intrinsic hydrophilicity, also have large molecular size such as the chiral monomers **4**, **8**, **10**, and **13** used in this study. It is likely that this difficult transfer of monomers of this type is the reason for the lack of previous data on the use of the templated suspension polymerization of chiral monomers for the direct preparation of CSPs.

Our new monomers **4** and **8** are quite soluble in the mixtures of cyclohexanol and 1-dodecanol that we typically use as the porogenic solvents for the preparation of porous beads from methacrylate-based monomers. Variation in the percentages of these two alcohols can be used to tune the porous properties of the beads. However, the swelling of the polymerization mixture into the activated templates becomes difficult once the 1-dodecanol content of the porogenic mixture exceeds 60%, regardless of whether dibutyl phthalate or 1-chlorodecane were used as the activator in the first swelling step. Table 1 summarizes the results of a series of polymerizations. It is worth noting that the contents of incorporated chiral monomers are close to those calculated from the composition of the polymerization mixture.

Although branched monomers **10** and **13** also dissolve in some mixtures of cyclohexanol and 1-dodecanol, the use of this porogenic system did not lead to porous beads from these monomers. Monitoring of the swelling process by optical microscopy revealed that these emulsified highly viscous mixtures only transfer very slowly into the activated templates. This is likely to result from the unfavorable solubility parameters and the large size of this branched monomer. Therefore, we tested other solvent mixtures as potential porogens and found that the pair toluene–cyclohexanol had both the boiling point and solubility parameters suitable for the preparation of beads with controlled properties from monomers **10** and **13.** Once again the data in Table 2 show that the contents of chiral monomer incorporated into the beads are a close match to the calculated values.

3.3. Characterization of chiral stationary phases

3.3.1. Porous properties

Table 1 also summarizes the porous properties of the CSPs derived from our monomers. CSPs 1–5 prepared using the same ratio of porogen to monomers have similar pore size distributions with most of the pores in a range of 10–25 nm. They differ only in their overall pore volume, and, consequently in their surface area. Both pore volume and specific surface area of the CSPs based on monomer **4**

Table 1
Composition of polymerization mixtures for the preparation of macroporous polymer CSPs from chiral monomers **4** and **8**

	Polymerization mixture (vol%)[a]				Loading (mmol/g)		S_p[b] (m^2/g)	V_p[c] (ml/g)
	Chiral monomer	EDMA	Cyclohexanol	1-Dodecanol	Theory[d]	Found[e]		
Monomer **4**								
CSP1	20	20	54	6	1.22	1.07	2.2	0.10
CSP2	20	20	48	12	1.23	1.08	5.4	0.12
CSP3	20	20	45	15	1.22	1.00	40.0	0.15
CSP4	20	20	42	18	1.22	1.07	81.2	0.21
CSP5	20	20	36	24	1.23	1.05	108.3	0.37
CSP6	28	12	36	24	1.66	1.55	5.0	0.26
CSP7	24	16	36	24	1.44	1.37	100.5	0.46
CSP8	16	24	36	24	0.87	0.80	173.8	0.50
CSP9	18	18	38.4	25.6	1.19	1.10	143.3	0.76
CSP10	16.5	16.5	40.2	26.8	1.22	1.10	170.0	0.91
Monomer **8**								
CSP11	18	18	57.6	6.4	0.88	0.83	7.1	0.101
CSP12	18	18	51.2	12.8	0.85	0.79	7.1	0.119
CSP13	18	18	44.8	19.2	0.80	0.80	7.1	0.116
CSP14	18	18	38.4	25.6	0.84	0.78	56.0	0.315

[a] Each polymerization mixture also contained azobisisobutyronitrile (1 wt.% with respect to monomers).
[b] Specific surface area calculated from nitrogen adsorption isotherm using BET equation.
[c] Pore volume determined by mercury intrusion porosimetry.
[d] Content of the functional monomer calculated from composition of the polymerization mixture.
[e] Calculated from the elemental analysis of nitrogen.

increase with the content of 1-dodecanol in the porogenic mixture. CSPs with larger pores as well as higher surface areas such as CSPs 9 and 10 are obtained by increasing the ratio of porogens to monomers. As shown in Fig. 2, these CSPs also contain 10–25 nm pores. In addition, they also feature larger pores in the size range of 25–70 nm. The porosimetric measurements indicate that these

Table 2
Composition of polymerization mixtures for the preparation of macroporous polymer CSPs from chiral monomers **10** and **13**

Sample	Polymerization mixture (wt.%)[a]				Loading (mmol/g)		S_p[b] (m^2/g)	V_p[c] (ml/g)
	Chiral monomer	EDMA	Cyclohexanol	Toluene	Theory[d]	Found[e]		
Monomer **10**								
CSP15	21.5	23.5	0	55	1.20	0.83	91.6	0.51
CSP16	21.5	23.5	11.8	43.2	1.20	0.85	91.7	0.51
CSP17	21.5	23.5	23.2	31.8	1.20	0.90	74.0	0.47
CSP18[f]	21.5	23.5	23.2	31.8	1.18	1.10	75.0	0.43
CSP19[f]	21.5	23.5	11.8	43.2	1.15	1.04	24.8	0.18
Monomer **13**								
CSP20	21.5	23.5	11.8	43.2	0.56	0.55	195.0	0.70

[a] Each polymerization mixture also contained azobisisobutyronitrile (1 wt.% with respect to monomers).
[b] Specific surface area calculated from nitrogen adsorption isotherm using BET equation.
[c] Pore volume determined by mercury intrusion porosimetry.
[d] Content of the functional monomer calculated from composition of the polymerization mixture.
[e] Calculated from the elemental analysis of nitrogen.
[f] Swelling time was extended to 2 days.

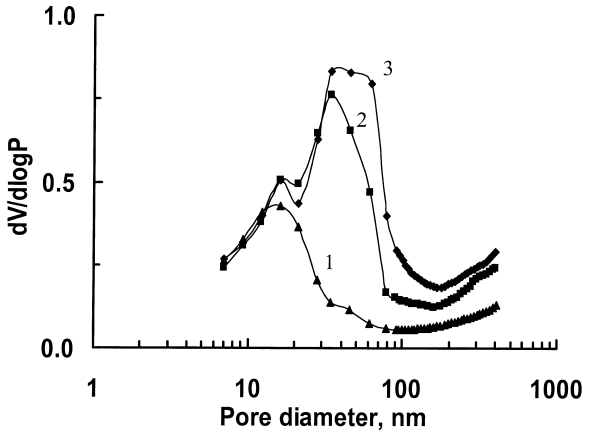


Fig. 2. Pore size distribution profiles for CSPs prepared from monomer **4** using polymerization mixtures with porogen to monomer ratios of 60:40 (curve 1, CSP 5), 64:36 (curve 2, CSP 9), and 67:33 (curve 3, CSP 10).

macropores contribute to the steep increase in the total pore volume.

CSPs 11–14 prepared from monomer **8** possess a longer linker arm between the chiral selector and the methacryloyl moiety. Although the porous properties of these beads are similar to those of CSPs derived from monomer **4,** both the specific surface area and the pore volume are much lower. This is likely to result from the differences in the phase separation process that occurs during polymerization and suggests that the cyclohexanol/1-dodecanol porogenic mixture is not well suited for the preparation of beads with ideal porous properties.

In contrast, toluene was used as the co-porogen with cyclohexanol for the preparation of CSPs 15–19 using monomer **10** containing a branched linker. Surprisingly, wide variations in the ratio of toluene to cyclohexanol from 100:0 to 60:40 only has a small effect on pore size, pore volume, and specific surface area (Table 2). The pore size distribution is broad and covers a range of 7–40 nm, with almost identical pore volumes (0.47–0.51 ml/g), and similar surface areas (70–90 m^2/g). A dramatic decrease in these values is observed only once the toluene content in the porogenic mixture with cyclohexanol drops below 20%.

Another variable that enables the control of porous properties of our beads is the percentage of ethylene dimethacrylate in the polymerization mixture. Table 1 shows the properties of CSPs 6–8 prepared from monomer **4** with different amounts of crosslinker. The pore size distribution profiles of CSPs 7 and 8 shown in Fig. 3 are similar and the beads are characterized by both large pore volumes and high surface areas. In contrast, CSP 6, crosslinked with only 30% ethylene dimethacrylate, has small pore volume. This indicates either that the phase separation during polymerization is poor and the pores do not form or that the porous structure is not sufficiently rigid and the beads collapse during drying.

A disadvantage of using variations in the percentage of crosslinking to control porous properties is that it leads to a change in polymer composition. The higher the percentage of crosslinking, the lower the content of the chiral monomer. Therefore, this approach is not suitable for the preparation of beads with a high content of functional monovinyl monomer.

3.3.2. Selector loading

Table 1 shows that more than 90% of the monomer **4** is incorporated in the CSPs according to the results of elemental analysis for nitrogen. The maximum selector loading we achieved is 1.37 mmol/g. This is almost twice the loading capacity of CSPs prepared earlier through modification of existing beads with a selector [24]. Table 2 also shows that the increase in size of the monomer results in its

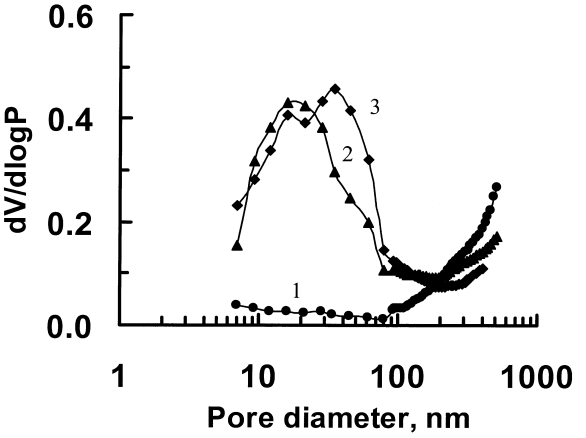


Fig. 3. Pore size distribution profiles for CSPs prepared from monomer **4** using polymerization mixtures containing 30 (curve 1, CSP 6), 40 (curve 2, CSP 7), and 60% (curve 3, CSP 8) of ethylene dimethacrylate.

much slower transport from the emulsion into the activated templates. This leads to larger difference between theoretical and experimental percentages of monomer **10** incorporated in CSP 15–17 prepared using standard reaction procedure. However, extending the duration of the second swelling step to 48 h enables an almost complete transfer of the monomer into the activated templates. As a result, the loading found for CSP 18 and 19 is in good agreement with the calculated values.

3.4. Evaluation of enantioselectivity

3.4.1. Initial screening

A batch approach involving the mixing a solution of racemic analyte with the CSP is a very simple method to screen the CSPs for enantioselectivity. This approach was recently used by Welch to screen libraries of CSPs [25,26]. It enables a rapid evaluation of CSPs using only a very small volume of beads, much less than is needed to pack an analytical column. In our implementation, less than an equimolar amount of *N*-(3,5-dinitrobenzoyl)leucine diallylamide racemate in 80:20 dichloromethane/hexane solution were equilibrated with beads containing selector functionalities, followed by chromatographic determination of the fraction of both enantiomers that remained in the supernatant liquid. While the peak areas for both enantiomers in the initial racemate solution is equal, the percentage of L-enantiomer in the supernatant liquid after the equilibration was always smaller than that of the D-enantiomer. The ratio of these two peak areas could then be used as a measure of enantioselectivity of the CSP. The larger the ratio, the higher the enantioselectivity.

The interaction of both enantiomers with the CSP is a dynamic process that reaches its thermodynamic equilibrium. It depends on the difference in the free energies $\Delta(\Delta G_i)$ of the two diastereomeric complexes formed by interaction of the selector with each of the two enantiomers and the concentration of the analyte in the system. The effect of concentration is eliminated by determining the percentages of enantiomers in the supernatant liquid using racemate solutions with different concentrations. The ratio of peak areas reaches its maximum at very low concentrations of the analyte and then levels off. We use the highest value of enantiomeric ratio observed at the low concentration of racemate at which the curve breaks to characterize each CSP. Once screening was completed, the most promising CSPs were prepared on a larger scale, packed in columns, and re-evaluated using standard chromatography.

3.4.2. Effect of the linker

The linker affects the chiral separation in three ways through both its length and chemistry: (i) it contributes to the surface polarity, (ii) it may introduce nonspecific interaction, and (iii) it improves the accessibility of the selector moiety [27,28]. For the preparation of one series of CSPs, we used a polyester linker with a chemistry similar to that of the polymer bead matrix to minimize the effect of the first two factors. This allowed us to focus our study solely on the effect of spacer length. The linker of monomer **8** is eight atoms longer than that of monomer **4**. Fig. 4 shows that the enantiomeric ratios of 1.75 and 2.02 were obtained for CSP 3 and CSP 14 prepared from monomer **4** and **8**, respectively. Indeed, the longer spacer appears to improve accessibility of the selector for its interaction with the enantiomers, leading to an increase in selectivity.

3.4.3. Effect of selector multiplicity

Two decades ago, Pirkle observed an enormous increase in selectivity factors for analytes in which

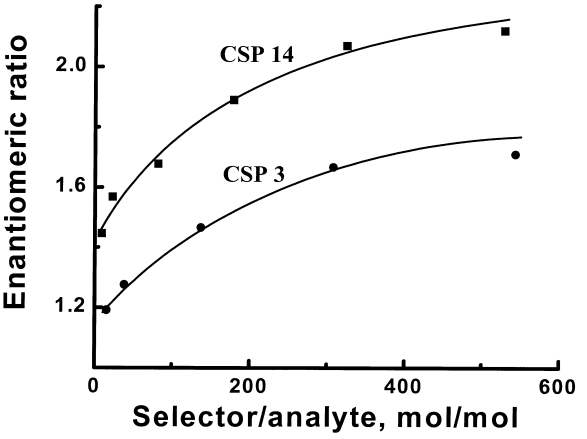


Fig. 4. Effect of linker length on selectivity of chiral stationary phases CSP 3 and CSP 14 measured by batch method and calculated as the ratio of peak areas of enantiomers in the supernatant after equilibration with solutions of racemic *N*-(3,5-dinitrobenzoyl)leucine diallyamide varying in concentrations.

two chiral moieties were linked through an alkyl chain [29]. The same author [30] also postulated in the late 1970s the principle of reciprocity: ''If a molecule of a chiral selector has different affinities for the enantiomers of another substance, then a single enantiomer of the latter will have different affinities for the enantiomers of the identical selector''. Therefore, it may be expected for polymeric beads that doubling the chiral moieties of the selector may also afford CSPs with very high selectivities. A preliminary short study we carried out using a multistep modification of preformed beads to prepare such twin selector indicated that CSPs with branched aliphatic linkers can improve the enantioselectivity [31]. However, the very low content of selector in these CSPs and the lack of suitable characterization did not allow us to adequately quantify the effect. In contrast, chiral monomers can be prepared as well-defined single compounds and their use enables incorporation of a higher percentage of selector into the beads. Therefore, we synthesized chiral monomer **10** in which two proline-based selectors are linked together through a 13-atom long ester linker itself linked at its center to the polymerizable methacrylate unit via a short space. CSP 18 prepared from this monomer was packed in a column and tested for enantioselectivity using several racemic analytes. For comparison, simultaneous testing was carried out with CSP 4 prepared from monofunctional monomer **4** and with properties such as overall selector loading, surface area, and pore volume similar to those of CSP 18. The results summarized in Table 3 document the positive effect of the interlinked twin selectors since CSP 18 exhibits 15–25% higher selectivity factors. This may indeed indicate a synergistic effect of the selectors attached to a branched linker. More importantly, we only observed a small increase in the retention of the less retained analyte while the retention of the more retained enantiomer almost doubled. This actually moves the eluted peaks farther apart, an effect valued mostly for columns engineered with high throughput in mind. Unfortunately, the increase in selectivity is by far not as dramatic as that reported by Pirkle.

In order to assess the effect of the branched linker itself, we also prepared CSP 20 using monomer **13** in which the identical linker carried only a single selector. Rapid microscale testing of the enantioselectivity illustrated in Fig. 5 affords enantiomeric ratios of 2.10 and 1.85 for closely matched CSP 15 and CSP 20, respectively. Although the preparation method does not allow access to beads with exactly matched properties and the difference in contents of

Table 3
Retention factors k' and separation factors α obtained for separations of racemic compounds using CSP 4, CSP 18 and CSP19

Analyte[a]	k'_1	k'_2	α
CSP4			
16a	0.20	4.52	22.60
16b	0.21	4.45	21.19
17a	0.20	4.66	23.30
17b	0.19	4.24	22.32
CSP18			
16a	0.29	7.81	26.93
16b	0.29	7.48	25.79
17a	0.31	7.75	25.00
17b	0.30	7.94	26.47
CSP19			
16a	0.26	5.03	19.35
16b	0.26	4.67	17.95
17a	0.28	5.07	18.10
17b	0.26	4.94	19.00

Chromatographic conditions: column: 150×4.6 mm I.D.; mobile phase: 20% hexane in dichloromethane; flow-rate: 1.0 ml/min; void marker: 1,3,5-tri-*tert*.-butylbenzene; UV detection at 254 nm.

[a] For structures of the analytes, see Fig. 1.

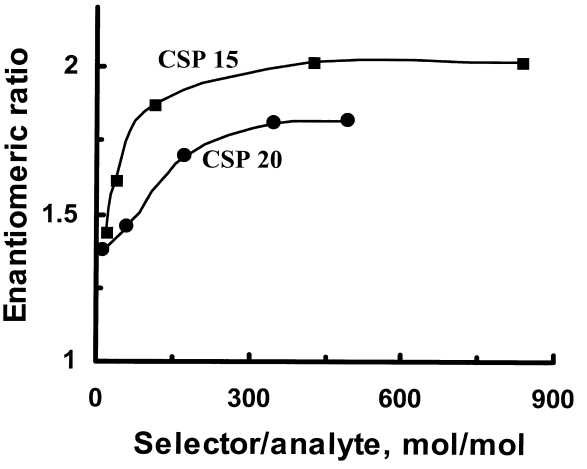


Fig. 5. Effect of linkers in CSP 15 and CSP 20 on enantioselectivity. Analyte: racemic *N*-(3,5-dinitrobenzoyl)leucine diallyamide.

the selector moieties may also contribute to the improved selectivity, this indicates again the positive effect of using twin chiral moiety on the selector for chiral recognition.

3.4.4. Effect of surface area

Macroporous polymers consist of an array of interconnected microglobules with a large number of voids (pores) between these globular units. Since the microglobules are highly crosslinked, it is expected that some of the functionalities may be buried within the impervious highly crosslinked polymer. Therefore, only those functionalities located at the vicinity of the pore surface would be readily accessible for the enantioselective separation process. Obviously, an increase in the specific surface area of the beads results in a higher proportion of exposed functionalities. As a result, the actual number of selector moieties participating in the separation process may differ significantly from the number determined by elemental analysis.

The effect of surface area on enantioseparation is well demonstrated in a comparison of CSP 4, CSP 9 and CSP 10 that have similar properties except for their specific surface area. Indeed, the saturation curves shown in Fig. 6 have different shapes for each of these CSPs and the enantiomeric ratio is the lowest for CSP 4 with the smallest surface area. In contrast, a less significant difference is observed for the CSPs with high specific surface areas. This is not completely surprising, since for polymers with equal pore volume, an increase in the surface area is typically accompanied by a decrease in the pore size. Therefore, some of the pores that contribute to the rather high surface area of almost 200 m^2/g in CSP 10 may no longer be accessible to the analyte molecules. As a result, only a fraction of the selector functionalities are accessible despite the fact they are located at the surface of the beads.

The effect of a moderate increase in surface area is also demonstrated with CSP 18 and CSP 19, both based on monomer **10**. These two CSPs have a similar content of selector as determined by elemental analysis but they differ in their specific surface areas that reach 24 and 75 m^2/g, respectively. Fig. 7 shows a large increase in retention of the more retained enantiomer while almost no difference in elution time is observed for the opposite enantiomer. Table 3 shows that the increase in enantioselectivity with the surface area is characteristic of all of the analytes tested.

3.5. High throughput separations

An increase in throughput of chromatographic separations can simply be achieved using larger columns scaled to the desired size operating under the conditions typical of analytical scale separations. This approach is investment-intensive but does not

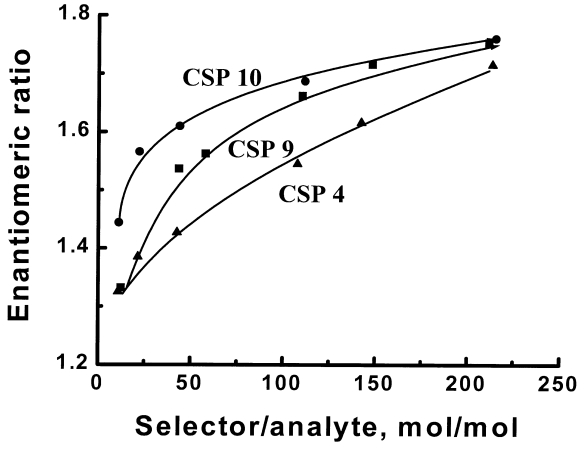


Fig. 6. Enantioselectivity of CSP 10, CSP 9, and CSP 4 differing in specific surface areas.

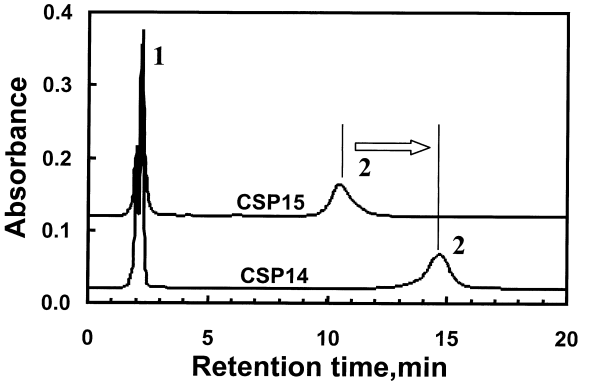


Fig. 7. Separations of racemic *N*-(3,5-dinitrobenzoyl)leucine diallyamide using columns packed with CSP 14 and CSP 15 differing in specific surface areas. Column: 150×4.6 mm I.D.; mobile phase: 80:20 dichloromethane–hexane; flow-rate: 1.0 ml/min; detection at 254 nm.

require any specifically developed CSPs. In contrast, processes that involve smaller columns operating under overload conditions enables high throughput operations at lower capital costs. These processes benefit from use of tailored CSPs with both high separation factors and high loading capacities. These critical properties depend both on the chemistry and the selector loading of the CSP. Since the direct polymerization of chiral monomers is well suited for the preparation of beads containing high percentages of selector moieties, the approach is attractive for the preparation of CSPs well suited for large-scale chromatography. Fig. 8 shows the touching band enantioseparation of 10 mg of racemic dinitrobenzoylleucine diallylamide using an analytical 150×4.6 mm I.D. column packed with CSP 14. A simple calculation reveals that even with such unoptimized system, 1.2 kg per day of the racemate could be separated using 1 kg of this CSP packed in a column and run in standard chromatographic mode. The purity of the separated enantiomers was assessed by collecting two fractions of the eluent cut at the valley between the peaks, then injecting them separately in the same column. Fig. 9 shows the chromatograms. The first fraction contains only the desired D enantiomer and a small amount of unidentified impurity. In contrast, a small peak of the D-enantiomer

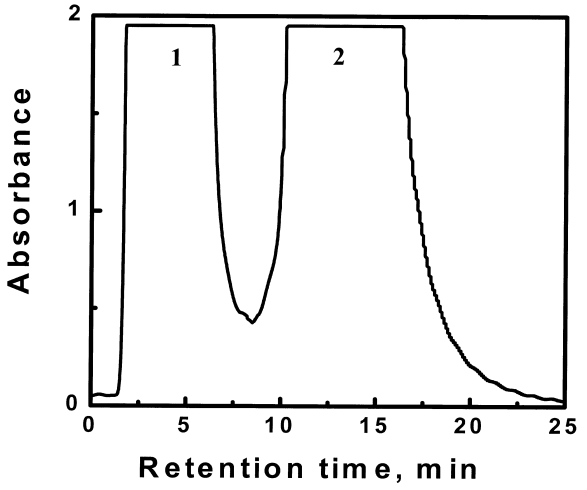


Fig. 8. Separation of racemic *N*-(3,5-dinitrobenzoyl)leucine diallyamide under overload conditions using CSP 14. Column: 150×4.6 mm I.D.; injection: 10.0 mg racemate; for separation conditions, see Fig. 7.

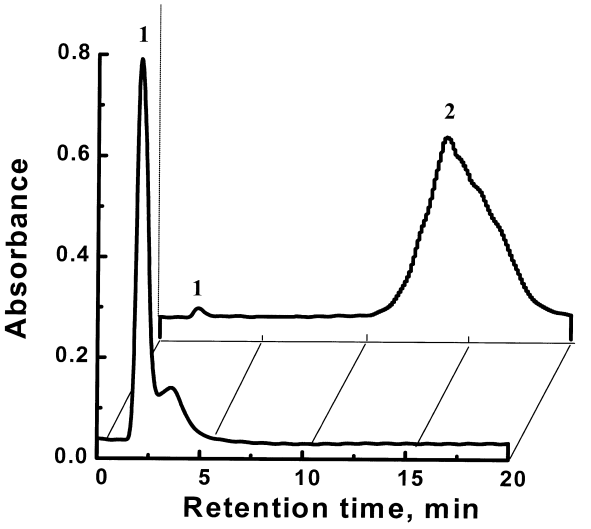


Fig. 9. Assessment of purity of the separated enantiomers collected in the preparative separation shown in Fig. 8.

representing about 0.5% of the total peak areas is visible in the trace together with a large peak of the L-enantiomer.

4. Conclusions

The preparation of CSPs using the direct polymerization of chiral monomers proved to be viable and attractive compared to the more common approach that includes the preparation of beads followed by their functionalization. In contrast to the latter, the use of chiral monomers leads to CSPs with well-defined functionalities and enables the preparation of monodisperse beads that can contain a relatively high percentage of the desired chiral moieties. This technique also allows the preparation of CSPs from specially designed monomers with a broad structural variety of both chiral selectors and linkers. To demonstrate the concept, we have chosen only a single selector linked to a methacrylate unit. However, linkers with a variety of new chemistries and architectures were used to connect these two components and their effect on the chiral recognition evaluated. The preparation of chiral monomers can be easily combined with screening of combinatorial libraries of potential selectors for chiral recognition in order to accelerate discovery of selectors targeted

towards a specific racemate. This combination appears to be a valuable tool for the preparation of CSPs useful in large-scale high throughput enantioseparations.

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